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Original Articles.

THE FUNCTION OF THE SOLUBLE FERMENTS OF THE BLOOD IN INTRACELLULAR DIGESTION.

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METABOLISM COMPARED TO COMBUSTION.

The constituents of the food, viz., the proteids, fats, carbohydrates and salts are indifferent to oxygen outside of the animal organisms but readily combine with oxygen within the body. As a certain amount of heat is generated and a certain amount of energy liberated during the passage of the food-material through the body, the whole process has been rather crudely compared to a combustion, life to a flame and the body to a machine that burns the fuel that is fed to it. This conception is inaccurate, for it merely considers the most superficial manifestations of the life-process, the initial and the terminal stages of a most intricate process, the finer, intermediary mechanism of which we are just beginning to understand.

If we determine the quantity of oxygen inspired within a stated time-unit and the quantity of oxygen excreted (in gaseous, liquid or solid combinations) through the various emunctories of the body in the same time-unit, we will find that nearly 19 per cent. (that is, nearly one-fifth) more oxygen leaves the body than enters it in the inspired air. This can be explained only in one way, viz.: some of the oxygen (one-fifth) contained in the excreta must be derived from another source than the air we breathe and that other source can only be the food. We can go a step further and say that the oxygen present in the food must be torn out of its combinations by a process that is akin to fermentation in its broader sense, and that consists in cleavage of proteid, fat or carbohydrate molecules; this process, moreover, must occur without the intervention of free oxygen; must, in other words, be anaërobic and take place in a reducing medium.

As a matter of fact, it can be shown that the assimilation and dissimilation of food occurs in two stages, 1, an anaërobic stage, in a reducing medium in the absence of oxygen, in the interior of the cell; 2, an aërobic stage in the presence of free oxygen (hemoglobin) at the periphery of the cell, i. e., chiefly in the blood and lymph stream. Both processes, it appears, are inaugurated by the intervention of soluble, chemical, unorganized ferments that the cells secrete. So-called "vital" processes are only indirectly concerned in these metabolic changes; for the present we are still forced to use the term merely to designate the power of cells in two directions, viz.: 1, their power to build up active, functionating protoplasm from simpler, inert compounds; 2, their power to secrete enzymes with specific properties. Much that was heretofore considered "vital" has been robbed of this dignity by recent discoveries relating to the rôle of enzymes in "intracellular digestion."

The term "intracellular digestion" is employed in contradistinction to extracellular digestion to designate those processes of digestion or destructive metabolism that occur in all the tissues of the body other than the gastro-intestinal tract—in reality it does not even exclude the processes going on in the mucosa and walls of the digestive canal, but merely those that occur in its lumen. As the material contained in the mouth, stomach and intestine is not really within the body, intracellular digestion is essentially synonymous with general metabolism.

Leaving the fate of the inorganic constituents of the food out of our consideration, we will briefly study how the molecules of proteid, fat and carbohydrate material are rent asunder by the ferments, how the smaller torn-off molecules are recombined to form new compounds, how these are again disassimilated to simpler complexes until finally some unfortunate atoms got out of their orbit, so to say, become chained to oxygen and stumble into compounds that have to be eliminated.

THE THEORY OF RECONVERSION.

The fermentative changes occurring within the intestinal tract are essentially physical in character, i. e., the proteids, fats and amylaceous portions of the carbohydrates are hydrolyzed to compounds that can diffuse through animal membranes. The proteids are converted to albumoses and peptones (and in very small part to amido-products), the fats to fatty acids and glycerin, the starches to soluble sugars. That this hydrolysis is merely intended to enable the entrance of the original bodies into the organism proper is manifested by the fact that in their passage through the intestinal wall they are reconverted. Within and behind the intestinal wall, i. e., in the blood of the mesenteric veins and the lacteals we never, consequently, encounter peptone or albumose but only serum-albumin; the same in all probability applies to the fats; glycerin and fatty acids being recombined to neutral fat in their passage through the intestinal wall. Whether or not the sugars enter the blood and lymph directly is not positively determined; a great portion of these sugars certainly reaches the liver as glycogen and is deposited there as such.

This process of reconversion has recently been shown to be due to enzyme-action. It appears that the same ferments that can split fat, e. g., into glycerin and fatty acids possess the power of recombining glycerin

and fatty acids to neutral fat. This process also occurs *in vitro* by the action of lipase, the ordinary fat splitting ferment of the pancreas (steapsin). The discoverers of this "reversion" process determined, moreover, that lipase is concerned in bringing about a condition of chemical equilibrium between the fat molecule (and water) and the products of its hydrolysis; in other words, that it forms a certain proportion of fatty acids and glycerin if neutral fat is present in excess, but that it is also inversely capable of forming a certain proportion of neutral fat from glycerin and fatty acids provided the latter bodies are present in excess in the solution.

The one objection to this theory is the fact that we can not identify ferments in any other way than by their manifestations; we have, moreover, no way of isolating them from the organ-extracts in which they are dissolved. It is not impossible, therefore, that pancreatic extract, or even so-called "pure" lipase contains two ferments, the one that splits fats into glycerin and fatty acids, the other that recombines the two to fat. This objection is strengthened by the observation that a ferment other than pepsin or trypsin, namely, rennet, seems capable of reconverting albumoses and peptones to coagulable albumin. If egg-albumin or fibrin are digested with artificial gastric juice (pepsin-HCl) and the albumoses and peptones that are formed removed by dialysis (a process analogous to the removal of these products from the intestine by osmosis through the intestinal wall) and if to the dilute clear solution of albumose-peptone some rennet is added, a flocculent coagulate of albumin will reappear. As rennet is a normal constituent of the gastro-intestinal mucosa, it seems probable that this ferment is concerned in the regeneration of serum-albumin from albumose-peptone.

DIGESTION LEUCOCYTOSIS.

The question arises, where do these processes occur? In the mucosa, the muscularis, the serosa, the lymph or blood stream? Presumptive evidence is overwhelming in favor of the supposition that the leucocytes in the blood and lymph capillaries of the intestinal wall are in some way concerned in this process (digestion-leucocytosis). We will revert to the rôle of these cells as ferment-carriers later on and show that disintegration of leucocytes, as a rule, precedes the inauguration of many fermentation processes, a disintegration that probably precedes and is accompanied by a liberation of enzymes. We will also attempt to ascertain the source and origin of the ferments that the leucocytes harbor in their interior and that they carry to any portion of the body where they are needed to establish chemical equilibrium. All the ferments are so toxic when circulating freely and are still so universally present that we can only assume that mobile cells, like the leucocytes, bear them in a harmless form through the blood and lymph-stream to their ultimate destination.

Of the food-albumin that circulates in the blood and lymph stream as serum albumin only a very small proportion is built up directly into living protoplasm. The cell protoplasm seems endowed with considerable longevity, so to speak, for only 16 to 20 gr. of cell-protoplasm die in twenty-four hours—a remarkably small proportion in an organism weighing from 70,000 to 75,000 grams (70-75 Kg.). From these figures we know that only 16 to 20 gr. of the circulating serum albumin are directly incorporated into the cell body, are endowed with life and become living protoplasm to take the place of that moiety of protoplasm that dies and is cast off into the blood stream.

The great bulk of the albumin, fat and carbohydrate, is simply food for the cells, and the source of the energy they require. With the aid of the enzymes the cells secrete, they split the large molecules. This process occurs in two stages; the first stage is a reduction and like every reduction requires heat; the result is the formation of numerous compounds that possess greater affinity for oxygen than the original bodies; the second stage is an oxidation in which the chemical affinities are satisfied and heat is developed. The result is the formation of highly oxidized end-products that are excreted.

The whole process, then, is a vicious circle—the heat that is converted into chemical affinity is derived from the oxidative processes that satisfy the chemical affinity—once inaugurated, the process goes on indefinitely, provided sufficient potential energy is introduced with the food that can be converted into kinetic energy.

This chemism is altogether analogous to similar processes occurring in the inorganic world. In order to reduce oxid of potassium, e. g., with carbon, heat is required; the result is metallic potassium that possesses great affinity for oxygen; if potassium is oxidized again the same amount of energy is liberated that was originally required for the reduction. We need not postulate any specific "vital" force. The universal laws of the conservation of matter and of energy operate in the same way in the organic as in the inorganic world. Living plants and animals merely possess specific powers of combining and converting matter and of transmuting energy into specific forms of motion that we call vital.

It would lead us too far were we to follow the stepwise degradation of the albumin, fat and carbohydrate molecules in detail; it may suffice to study the principal products of the first anaërobic, reducing stage, and of the second aërobic, oxidizing stage, and to elucidate, as far as we are able, the ferment mechanism of these conversions.

ANALOGOUS PROCESSES IN BIOLOGY.

A clear insight into these processes can be gained by drawing analogies with similar bio-chemic reactions that are carried on by unicellular micro-organisms. The animal body, after all, is merely a conglomeration of numerous unicellular micro-organisms, in which each cell is a special micro-laboratory and leads an independent existence while at the same time contributing its share to the maintenance of the whole. Unicellular organisms are so small compared to complex multicellular organisms and the metabolic changes they bring about in the media they grow in are so colossal in comparison to their bulk, that we are apt to overlook the fact that these metabolic processes are merely incidental, and certainly subservient, to the biologic needs of the cells. The character of the protoplasm of bacteria and of our body cells, moreover, is different only in distribution and not in character.

As a prototype of anaërobic life, as an illustration of the first *reducing* stage of metabolism, the life processes of *tyrothrix urocephalum*, a bacterium that has its natural habitat in putrefying milk, may be studied. This germ, when grown on milk, attacks the proteids of the milk alone and leaves the fats and carbohydrates intact. If oxygen is excluded, it splits the proteid molecule; combines some of the carbon with some of the oxygen, and generates CO_2; combines hydrogen with oxygen and forms water; secretes a proteolytic enzyme that converts other portions of the proteid molecule

into peptones and then into urea, fat, ammonia compounds, tyrosin, a variety of ptomaines and certain intermediary acids. This germ requires some oxygen, but it can derive it from the oxygen atoms contained in the proteid molecule—the oxygen is needed, not for the metabolic processes that are carried on by the enzyme the bacterium secretes, but for the maintenance of the scanty life-processes of the cell.

Altogether analogous processes are carried on by the cells of the animal body. If a piece of muscle is kept in a vacuum at body temperature CO_2 will be developed and water will be formed. There is evidence to show that the fats and carbohydrates are not disassimilated in this first stage, but that the proteid molecule alone is attacked as in tyrothrix and that it is converted into urea, glycogen and fat, ammonia products, leukomaines and a large variety of intermediary bodies. These processes have been studied particularly in the liver, but it can readily be shown that similar processes occur in other organs of the body; it is probable that this form of activity is a universal property of all our cells.

INTERMEDIARY PRODUCTS ARE TRANSIENT.

During life all these intermediary products are immediately oxidized so that it is a very difficult task to detect them. In certain diseases and intoxications, however, in which the aëration of the blood is interfered with, in dyspneic states, in the agonal stage, or immediately after death when the individual cells still live although the blood no longer circulates, these products accumulate very rapidly in the tissues and can be detected there. The reducing properties, finally, of nearly all the organs of the body have been repeatedly demonstrated by coloring methods (Ehrlich) and by feeding- and injection-experiments with iodates, indigo, certain pigments, etc.

The end products of this first stage are carbon dioxid (CO_2), water, urea, creatin, etc., on the one hand, and a large number of intermediary products on the other. The former must be considered "rests," waste products, so to say, that have to be eliminated. Many of the intermediary products travel to the liver and are there forced into new combinations; some form urea, some uric acid, etc., but the majority of the fats, carbohydrates, hydrocarbons, amido acids, together with the original fats and carbohydrates of the food undergo oxidation in the blood stream.

FUNCTIONS OF VARIOUS FERMENTS.

Whereas we have so far been unable to demonstrate experimentally that the cells of our body, like certain bacteria, of which tyrothrix urocephalum is the prototype, secrete enzymes that bring about the processes of proteolytic anaërobic fermentation that we have discussed, we are able to show experimentally that the second, oxidative stage of dissimilation of fats, carbohydrates, etc., is due to the action of numerous soluble ferments that are present in the blood and lymph.

If blood or lymph is allowed to flow directly into a sterile vessel and the vessel allowed to stand for a number of hours at body temperature a progressive loss of the blood sugar will be observed. Occasionally a slight increase in the titre for dextrose will be noticed during the first half hour, followed, however, by a rapid decrease during the hours following. This preliminary increase only occurs when the blood contains some glycogen, and is explained by a preliminary conversion of glycogen to dextrose. Here, then, we see the action of two ferments, one that can convert glycogen to sugar (an amylolytic ferment), one that can destroy sugar (a glycolytic ferment). We can go still further. If we analyze the sugar that is formed from glycogen in the blood, we will find that it consists in part of maltose (a double sugar), in part of dextrose (a simple sugar). By allowing blood to flow into alcohol and leaving the coagulate that forms in contact with alcohol we destroy the power of the blood to form dextrose, and only maltose is formed from glycogen. In other words, the blood contains both a maltase and a glycase, i. e., a maltose- and a dextrose (glycose)-forming ferment, the latter being destroyed by contact with alcohol (Bial).

If chylous fat is mixed with blood or lymph and if the mixture is allowed to stand at body temperature some of the fat will be destroyed and converted into unidentified bodies (possibly fatty acids and glycerin) that, in contradistinction to fat, are in large part insoluble in ether and are dialysable.

Another ferment called oxidase is present in the blood and lymph that possesses the power of oxidizing a number of organic substances; besides, pepsin and trypsin in appreciable quantities, not to speak of the so-called fibrin-ferment, are all found in the blood and lymph.

ORIGIN OF FERMENTS IS OBSCURE.

The question arises, in what constituent of the blood do we find these ferments, in the serum, the red or the white blood corpuscles; where do they come from; what is their ultimate fate?

If the blood is centrifuged and serum and corpuscles examined separately, it will be found that the serum possesses neither diastatic, glycolytic, nor lipolytic properties; an extract made from the corpuscles, on the other hand, does possess these properties to a marked degree. If a piece of one of the veins of a large animal is ligated in two places and excised, then suspended in the ice chest for twenty-four hours, coagulation of the blood does not occur and under favorable conditions red corpuscles, white corpuscles and serum can be aspirated separately. It will be found that the ferment power is inherent in the white and not the red cells. When we consider in addition that the lymph possesses still greater glycolytic and lipolytic powers than the blood, when we remember the phagocytic action of the leucocytes towards all poisons (and the ferments are very toxic) we will agree that these ferments are carried by the leucocytes. As a matter of fact leucocytes possess distinct digestive powers towards bacteria. Finally, if chemotactic bodies are added to the blood that promote rapid disintegration of leucocytes, the ferment-powers of the blood are more rapidly developed—all of which seems to justify the conclusion that the ferments are carried by the leucocytes and that disintegration of leucocytes must precede the liberation of the ferments.

Some investigators claim to have found the ferments in the serum, and as a matter of fact they are frequently there—but only after the leucocytes have begun to degenerate, an accident that can hardly be avoided when the blood is subjected to such manipulations as defibrination, centrifugation, etc. The origin of these blood ferments is obscure; there are three possibilities. They can either be formed in the leucocytes themselves, they can be formed in all or many of the tissues and organs of the body, i. e., by protoplasm in general, or they can be derived from internal secretions of the digestive glands. It is altogether impossible at present

to decide this question. In view, however, of the tendency to specialization that becomes operative in any large congregation of individual cells, the latter view is the most plausible—the digestive glands would then furnish enzymes both for extra- and intra-cellular digestion.

FINAL DESTINATION OF FERMENTS.

Whether the blood ferments after they perform their function are destroyed, eliminated, re-inclosed in leucocytes, arrested in the digestive glands to be re-excreted, or finally are held by such dis-intoxicating organs as the suprarenals, the thyroid and the liver it is impossible to say. As the urine nearly always contains some of the ferments, as they are nearly always present in the liver, and as they certainly must suffer the ultimate fate of all the other constituents of the body, it is probable that all these processes can and do occur.

It has been objected that the ferments are present in such minute quantities in the blood that they can not possibly be credited with an important rôle in intracellular digestion; but this objection is invalid. In the first place they are present in minimal quantities in the extracts we prepare because they are not intended to be present in large quantities at any time nor to be copiously excreted like the digestive ferments. When we prepare our extracts we destroy the cells and force the ferments into solution; besides, we work with small quantities of blood and consequently very small numbers of leucocytes. In studying the action of such an extract, moreover, we see only a small portion of the power that would have been expended had the cells remained intact, had the blood remained alive. I can best illustrate this by comparing the action of the ferment extracted out of a kilo of yeast-cells and the action of a kilo of living yeast-cells. The ferment solution will develop less power by far than the living cells, as in the latter new ferment is continuously being formed; in other words, the action of the ferment accumulating during an hour is only a small proportion of the hourly action of the cells. The ferment solutions from the blood only contain the remnant that is not utilized during life.

Furthermore, nearly all ferment extracts from the blood are made after defibrination or alcohol-coagulation, and it is a well-known fact that fibrin and coagulates retain ferments with a tenacity that makes it almost impossible to separate the two—in this way, too, a large proportion is lost.

From very recent investigations it appears that the ferments are chemically (if they are chemical entities at all!) nucleo-proteids, i. e., derivatives of the cell-nuclei. They are in general so akin to protoplasm both i their chemical reactions and their behavior to various physical and chemical agencies that they must be considered closely related to living protoplasm. As a matter of fact all protoplasm has some ferment-power—ferments seem to be fragments of protoplasm, and living protoplasm can do all that ferments can do and more besides in other directions, viz., it has the power of regeneration (see above) and of "assimilation."

REGENERATION OF PEPSIN BY FIBRIN.

Some investigations that I am at present engaged in seem to point to a still closer connection between ferments and living protoplasm even in its most "vital" properties. If 0.01 gr. of pepsin is dissolved in 95 c.c. of 2.75 per cent. HCl and 5 gr. of fibrin or egg-albumin added, the fibrin will be digested within twenty-four hours and the fluid will become clear; of this fluid 10 c.c. are mixed with 85 c.c. of the HCl solution and 5 gr. of fibrin are added again; this, too, will be digested. This process of dilution can be repeated many times and the digestion of fibrin will proceed almost as rapidly as in the beginning; not until the 9th or 10th dilution will an appreciable retardation become noticeable. If we calculate the dilution, we will find that 1 part of pepsin seems capable of digesting many million parts of fibrin. If these same dilutions of pepsin solution are made without adding fibrin and permitting digestion for twenty-four hours, it will be found that no solution of the fibrin occurs after the dilution is greater than 1 to about 30,000 to 50,000. (This applies to the particular pepsin I am using). The whole process resembles a regeneration of pepsin by contact with fibrin—in other words, a growth in a suitable medium. In the absence of pabulum no growth occurs.

If these findings can be verified and amplified and extended to other ferments, they promise to carry us several steps forward into the borderland that lies between dead and living protoplasm.

EPILEPSY, ITS ETIOLOGY, PATHOLOGY AND TREATMENT BRIEFLY CONSIDERED.*

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In the United States one person in every 500 suffers from epilepsy. In continental Europe the proportion is thought to be somewhat less, though this may be an error due to imperfect statistics; in England the ratio is about the same as in this country.

Epilepsy is one of the oldest diseases of which we have any record. Five centuries before Christ it was described by Greek physicians with much clinical accuracy.

It may be in the infant at birth, or develop immediately after, or its coming may be deferred until man has passed the milestone of life that marks to his credit threescore years and ten.

It comes irrespective of race, environment, occupation, social condition or position; affecting alike the poverty-stricken dweller of the tenement along with the rich who live in palaces.

American statistics show it to be slightly more common in males than in females, while European observers find the opposite true. In some the attacks may occur daily for years and yet produce only the slightest enfeeblement of any of the mental faculties, while in others a few attacks may, in a year's time, largely destroy all the faculties of the mind, producing in the end epileptic dementia. Its seizures may develop with sudden, shock-like violence, striking its victim to earth in the twinkling of an eye, or they may come and go with only the blanching of the face and a momentary void in the operations of the mind. In the same individual we may witness its manifestations hundreds of times in the space of twenty-four hours, at one period of the disease, and we may see months or even years intervene between its paroxysms at another period of the disease. It has no distinctive or pathognomonic symptom, for we find its two most prominent ones—convulsions and the impair-

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